A review of coral reef restoration techniques

Erik H.W.G. Meesters, Sarah R. Smith, Leontine E. Becking

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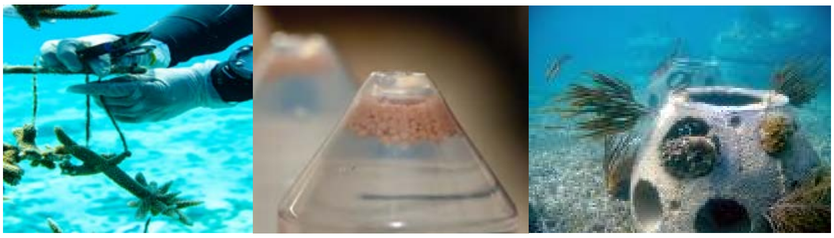

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P.O. Box 68
1970 AB IJmuiden
Phone: +31 (0)317 48 09 00
Fax: +31 (0)317 48 73 26
E-Mail: imares@wur.nl
www.imares.wur.nl

P.O. Box 77
4400 AB Yerseke
Phone: +31 (0)317 48 09 00
Fax: +31 (0)317 48 73 59
E-Mail: imares@wur.nl
www.imares.wur.nl

P.O. Box 57
1780 AB Den Helder
Phone: +31 (0)317 48 09 00
Fax: +31 (0)223 63 06 87
E-Mail: imares@wur.nl
www.imares.wur.nl

P.O. Box 167
1790 AD Den Burg Texel
Phone: +31 (0)317 48 09 00
Fax: +31 (0)317 48 73 62
E-Mail: imares@wur.nl
www.imares.wur.nl

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Summary

In this review the following three reef restoration techniques are discussed: 1. Coral gardening, 2. Larval seeding, and 3. Reef balls. These techniques are commonly used in the Caribbean and have widely different approaches. Coral gardening utilize the natural process of asexual reproduction through fragmentation to provide new coral clones for population growth. Healthy wild colonies are once clipped/fragmented and further grown (and cloned multiple times) in an underwater nursery and ultimately transplanted to the reef.
In contrast, larval seeding is based on the sexual reproduction of corals, where large amounts of coral eggs and sperm are collected in the field with subsequent fertilization in the lab. The coral recruits are then made to settle and grown in aquaria until a certain size, after which they are transplanted to the reef. Reef balls are artificial concrete structures designed to provide shoreline protection and sometimes shelter for fish, while at the same time providing substrate for natural recruitment and attachment of benthic organisms such as corals.

A general introduction to coral reproduction is provided to show how life history characteristics are used in restoration efforts and how these can affect the genetic variation within coral populations. The three approaches are compared based on:

1. Survival of fragments and larvae before transplantation to the reef.
2. Survival of transplants at the restoration site.
3. Introduction of exogenous material.
4. Indirect effects of coral restoration on the reef.
5. Genetic diversity.
6. Feasibility and effectiveness.

The main advantages of the production of colonies from fragments are that it bypasses the early larval stages where mortality is high and that new colonies can be grown completely in the field. Generally, the asexual reproduction technique demands less advanced expertise and the public outreach of this method is high because volunteers can easily be incorporated into the program. Furthermore, results become apparent relatively soon since the used species are relatively fast growing. However, there is the risk of creating populations with little genetic variability and the method is only applicable to branching coral species. Presently the method is mainly used for one single species, namely staghorn coral (*Acropora cervicornis*).

Larval seeding (sexual reproduction) is arguably the best method since it ensures natural genetic diversity and can be used with many species. The disadvantage with this method is that it demands a reasonably high level of expertise and takes more time than the asexual production of new colonies by fragmentation. Also a high percentage of new colonies is lost during the early stages. It is still mostly in the development phase.

Reef balls may increase fish biomass and protect shorelines, but their potential for coral reef restoration is judged to be limited due to the generally low levels of natural recruitment to these structures.

The restoration techniques suffer presently from a lack of independent scientific publications with good data to validate survival, regeneration, and growth rates of colonies in the different phases of the restoration program.

Different populations of the branching *Acropora* species can differ fundamentally in reproductive characteristics and may respond differently to environmental change. Their difference in strategy may

also be a result of adaptation to local environmental factors. All studies and protocols thus stress the necessity to adapt methods to specific locations and environments.

Consideration of genetic factors is essential because the long-term success of restoration efforts (depending on resilience of the populations) may be influenced by genetic diversity of restored coral populations. The use of molecular tools may aid managers in the selection of appropriate propagule sources, guide spatial arrangement of transplants, and help in assessing the success of coral restoration projects by tracking the performance of transplants, thereby generating important data for future coral reef conservation and restoration projects.

It is proposed to study genetic variation in the natural populations around the islands of the Dutch Caribbean and within the various restoration projects in progress. Additionally, it is recommended to assess survival, growth and regeneration of fragments ánd mother colonies in the field. We recommend to combine characteristics of the two main coral restoration techniques (fragmentation and larval rearing) to create a new hybrid approach to increase survival of sexually derived colonies and genetic diversity. In addition, the cost-effectiveness of the larval seeding method should be ascertained and compared with the fragmentation method.

We conclude by pointing out that reef restoration can only be successful if environmental conditions are adequate for survival and growth of coral colonies. This will mean that presently the selection of restoration sites with good environmental conditions is crucial. Thus, active management of anthropogenic stressors is a prerequisite for reef restoration — if a reef is not effectively managed and chronic stressors persist or develop, restoration will ultimately fail. Reef restoration must only be considered as complementary to management tools that address the wider causes of reef degradation.

1 Introduction

In the Caribbean, coral cover in shallow waters has been reduced from about 50% to about 10% over the last 30-40 years (Bak et al. 2005, Gardner et al. 2003, Jackson et al. 2014). The Caribbean island of Bonaire is a popular diving destination, as it is known for its well-developed fringing reefs and high biodiversity. The island's reefs are considered to belong to the best reefs in the Caribbean (Jackson et al. 2014). Yet coral cover has slowly, but steadily, declined from approximately 40% in 1973 to 20% in 2003 (Bak et al. 2005). Other researchers have compared the reef of Bonaire using shorter time series, but all studies conclude an overall decrease in coral cover and diversity and an increase in macro algal cover (summarized in Sommer et al. 2011).

There are predictions that one-third of all reef-building corals are at risk of extinction (Carpenter et al. 2008); 75% of reefs are highly threatened by the compounding effects of local stressors and factors associated with global climate change (e.g., ocean acidification, sea level rise, thermal stress, disease, cyclones) (Hoegh-Guldberg 1999; Baker et al. 2008; Veron et al. 2009) and as a consequence of local disease outbreaks. In the early eighties, White Band disease wiped out virtually all *Acropora cervicornis* (staghorn coral) on Caribbean reefs and seriously impacted *A. palmata* (elkhorn coral) (Gladfelter 1982). These corals dominated the shallow fringing reefs of Curaçao and Bonaire until the outbreak of the disease (Bak 1974, 1975, 1977; Duyl 1985). Especially *A. cervicornis* could be found all around Bonaire and Curaçao in extensive fields covering often hundreds of meters of the reef bottom (Duyl 1985). By 1985 more than 90% of these fields had disappeared. The decline of dominant coral species, has changed many coral reefs from complex three-dimensional living structures to flat stretches of seascape with much lower structural complexity in the shallows (Alvarez-Filip et al. 2009). The loss of structural complexity results in a major loss of reef function and structure leading to lower biodiversity of coral reef organisms, loss of fish biomass and greatly reduced buffering capacity for storm and hurricane damage and erosion.

Coral reef restoration has gained recent popularity in response to the steady decline of corals (Young et al. 2012). Reef restoration approaches actively attempt to restore damaged reef areas to their ''natural'' or ''original'' state after a disturbance by increasing live coral cover and species diversity through larval seeding or coral transplantation of whole adult or juvenile colonies, colony fragments from adjacent unaffected habitats or coral nurseries (Amar and Rinkevich 2007, Rinkevich 2005, Precht 2006). First, however, is the need for effective management of anthropogenic threats/stressors in addressing reef degradation. Subsequently, where management is effective but natural recovery occurs too slowly or is not likely to occur at all, active reef restoration may possibly assist in natural reef recovery (Edwards and Gomez 2007).

Young et al. (2012) conducted a literature review of various aspects of coral restoration techniques and conducted a questionnaire survey among 79 coral reef restoration practitioners: the most common participants were individuals associated with academic institutions and private organizations (39.3%), government employees (30.4%), or members of NGOs (18.9%). Throughout this report we make frequent reference to this review.

Three common reef restoration methods are currently applied in the Caribbean:

1. Coral gardening with asexually produced coral fragments.
2. Restoration with sexually produced recruits.
3. Reef Balls, concrete structures made to simulate reef structure and provide substrate for coral attachment and growth.

In this report we provide a description of each method and review the pro/cons using the following criteria:

1. Survival of fragments and larvae before transplantation to the reef;
2. Survival of transplants at the restoration site;
3. Introduction of exonogous material;
4. Indirect effects of coral restoration on the reef;
5. Genetic diversity;
6. Feasibility and effectiveness.

We also suggest further research to answer current gaps in our knowledge in the Dutch Caribbean.

A glossary (Appendix 1) for genetic terms is provided for the words in **bold.**

2 Introduction to coral reproduction and survival of recruits

Corals propagate sexually through the production of gametes as well as asexually through fragmentation (This leads to the generation of clones, colonies that are genetically the same) (Tunnicliffe 1981, Highsmith 1982, Bruno 1998). Fragmentation is caused by external physical disturbance and this is common in branching acroporids (Tunnicliffe 1981, Baums et al. 2006), as well as *Madracis* (Vermeij et al. 2007), *Porites* (Hunter 1993) and *Pavona* (Willis and Ayre 1985) but has also been reported for massive *Montastraea* species (Foster et al. 2007). Fragments have a higher chance of survival when they are large (Lirman 2000) so that dispersal is limited but, over time, **genets** can extend over tens of metres (Neigel and Avise 1983; Baums et al. 2006; Foster et al. 2007). As most corals are clonal organisms they may survive partial death of the tissue covering a coral skeleton (Fig. 3). The parts that do not die, may become separated over time and live on as different colonies (though they are genetically the same, e.g. Fig. 3). Dead parts of a coral may also become colonised by other organisms that grow on (or in) the coral and expand at the cost of the living coral tissue. If the invading organism is boring into the skeleton (e.g. boring sponges or boring molluscs) the coral skeleton may become weakened and the coral colony may fragment naturally at a certain moment in time.

Most recent coral reef restoration projects in the Caribbean have focused on the threatened (IUCN red list CR-critically endangered; US Endangered Species Act Threatened; SPAW protocol Annex II, CITES Appendix II) species *Acropora cervicornis* (Fig. 1) and *A. palmata* (Fig. 2), respectively commonly called Staghorn and Elkhorn coral.

Figure 1. Acropora cervicornis (Staghorn coral, photo Erik Meesters).

Figure 2. Acropora palmata (Elkhorn coral, photo Erik Meesters).

Both species dominated the Caribbean reefs before 1980 forming dense, three-dimensional thickets in intermediate (5–15 m) water depths, contributing significantly to overall reef growth, island formation, and coastal protection (Bak 1975, 1977). They were severely reduced during the early eighties, primarily due to a disease which is still not fully understood (Gladfelter 1982). The disease remains present and infected colonies can still be found. Both species are listed as critically endangered by the International Union for Conservation of Nature (IUCN). Species of the genus *Acropora* also have a high chance of success in asexual propagation and restoration due to their life history characteristics; both are the fastest growing species in the Caribbean and can quickly regenerate from damage (Bak 1983; Meesters et al. 1992).

Propagation by fragmentation is a common way of asexual reproduction for coral species that live in relatively shallow water such as the branching *Acropora palmata* and *A. cervicornis* (Figures 1 & 2). These species are prone to damage and breakage by waves, and fragmentation has become a part of their life history strategy. The degree to which these species are adapted to fragmentation is apparent in the rate with which they recover from physical damage (Meesters et al 1992, 1995). Williams et al (2008) estimate that only 11% of *A. palmata* colonies has a sexual origin in elkhorn patches in Florida.

From the enormous number of larvae produced over the lifetime of a coral, only a tiny fraction will survive, and grow to become larger coral colonies that recruit successfully into the adult population. Larvae are furthermore selective in their habitat choice, determined in part by the habitat of their parents (Carlon 2002, Baird et al. 2003, Vermeij et al. 2007). The survival of coral recruits increases exponentially with size (Bak and Engel 1979, Hughes 1984). Thus restoration success will increase when restoring larger coral colonies to the reef. Consequently, raising recruits or small coral colonies under controlled conditions till they have reached a more viable size or using coral fragments may strongly increase survival chances and the rate of success of restoration efforts.

For restoration by sexually derived propagules early survival and fast growth may be important characteristics, while for fragments that need to be broken off ‘mother’ colonies high regeneration capabilities and fast growth may be important.

3 Introduction to genetic variation

3.1 Genetic variation

The amount of variation in an organism's DNA is the combined product of past and present population processes. **Genetic variation** is a measure of the genetic differences within populations and species. Resilience of coral reefs relies strongly on the genetic variation within the organisms inhabiting and forming reefs. Due to the importance of preserving the genetic integrity of populations, strategies to restore damaged coral reefs should attempt to retain the genetic diversity of the disturbed population (Baums 2008, Shearer et al 2009). The variation in genetic material can be measured on different levels: individual, population, species.

3.2 Genetic consequences of asexual/sexual propagation

One of the consequences of asexual reproduction is that the number of individuals with the same genetic material (**genotype**) increases, thereby decreasing genetic variation and potentially increasing the species sensitivity to diseases. A genotype may occur several times in the form of clonal **ramets** in a population as a result of asexual replication. A ramet is an individual that develops from a genet or another ramet and is a product of asexual reproduction. A ramet is an exact genetic copy of its parent colony, and is thus considered a clone. Clones behave exactly the same under equal circumstances, which means they are equally strong, but also equally weak as their parent colonies.

While asexual reproduction through fragmentation can result in the expansion of local populations, this reproductive mode yields new colonies that are genetically identical to the parent (i.e., clones). Overreliance on asexual fragmentation limits the number of genetically distinct colonies, both at local scales and throughout the species range.

From the vast number of larvae produced by each coral through sexual reproduction, only a small fraction will recruit successfully into the adult population. Successful genotypes are then preserved through asexual reproduction, thereby increasing the relatedness of colonies on small spatial scales.

3.3 Genetic variation in natural scleractinian populations – site specific adaptation

Baums et al. (2006) have shown that within a species there are geographic differences in the contribution of reproductive modes to population structure and that these differences may be related to habitat and environmental characteristics. As a result different populations will likely respond differently to environmental change and may show different levels of resilience to a given location/environment.

The degree of clonality may vary over a species' range in accordance with the relative success of sexual and asexual recruitment. High genetic variation of structural species will promote resilience of ecosystems in the face of environmental extremes. Conversely, low genotypic diversity may indicate an asexual strategy to maintain resources during population decline (Baums et al. 2006).

A literature review by Shearer et al. (2009) provides a generalized estimate of genetic diversity (in terms of allelic richness) of natural scleractinian coral populations (Table 1). This provides a baseline of genetic variation in natural populations.

Figure 3. A large coral colony that has been fragmented into different disconnected parts, each part is now considered to be an individual (photo Erik Meesters).

Table 1. Genetic variation in natural populations of common corals from Shearer et al. *(2009) Genetic variation is described here as allelic richness (number of alleles per locus) and sizes of scleractinian coral populations in surveyed literature (multiple records for a species indicate data for different populations)*

Species	No. of populations	No. of loci	No. of alleles per locus per population (mean)	No. of colonies sampled per population (total sampled)	Reference
Acropora millepora	1	9	5–20 (8.7)	23 (23)	vanOppen et al. (2007)
	1	1	11 (11.0)	20 (20)	vanOppen et al. (2007)
A. nasuta	8	1	3–6 (3.9)	10–39 (216)	Mackenzie et al. (2004)
A. palmata	10	4	10–18 (14.4)	39–127 (818)	Zubillaga (unpub. data)
Favia fragum	2	15	2–16 (5.2)	45–48 (93)	Carlon and Lippe (2008)
Goniastrea favulus	1	5	2–10 (5.3)	32–44 (32–44)	Miller and Howard (2004)
Montastraea annularis	3	4	3–15 (9.8)	45–48 (146)	Foster et al. (2007)
M. cavernosa	1	5	9–17 (12.2)	58 (58)	Shearer and Coffroth (2004)
	10	5	6–16 (10.1)	21–56 (363)	Shearer (2004)
M. faveolata	4	6	4–29 (14.7)	150–216 (692–780)	Porto (Table S1)
Platygyra daedalea	1	5	4–11 (7.2)	50–80 (50–80)	Miller and Howard (2004)
Pocillopora damicornis	1	10	3–10 (5.6)	21 (21)	Starger et al. (2007)
	2	7	6–10 (7.9)	55–64 (119)	Shearer (Table S1)
P. meandrina	7	4	5–18 (10.2)	22–49 (257)	Magalon et al. (2005)
Porites astreoides	1	3	3–9 (6.0)	50 (50)	Shearer and Coffroth (2004)
	7	2	2–6 (3.9)	12–52 (192)	Shearer (unpub. data)
Seriatopora hystrix	1	10	3–14 (6.7)	51 (51)	Underwood et al. (2006)
	1	9	2–15 (5.7)	49 (49)	Underwood et al. (2006)
	10	3	5–16 (8.6)	11–32 (207)	Maier et al. (2005)

Most reef-building corals contain photosynthetic algae, called **zooxanthellae**, that live in their tissues. The corals are typically highly dependent on the algae with which they have an obligate relationship. The coral provides the algae with a protected environment and compounds they need for photosynthesis. In return, the algae produce oxygen, help the coral to remove wastes, and supply the coral with glucose, glycerol, and amino acids. **Holobiont** is the host plus all of its symbiotic microorganisms.

When studying the genetic variation of corals, it is therefore critical to also consider the genetic variation of the symbiotic **zooxanthellae.** Studies show that there is habitat-specific variation in zooxanthellate groups in corals (LaJuenesse et al. 2009, Baums et al. 2010). Several studies show local dominance of certain genets at specific sites or environments. Such studies have produced information on the performance of the specific combination of coral and zooxanthellate genotype present in the experimental units. Adaptation response of the **holobiont** to changing conditions, specifically rising seawater temperatures, has mostly been attributed to the zooxanthellate partner. Functional differences exist among taxa of zooxanthellae (Iglesias-Prieto and Trench 1997, Loram et al. 2007) and host–symbiont associations change predictably over depth gradients (mostly in the Caribbean; Frade et al. 2008; LaJeunesse 2002; Warner et al. 2006). When environmental conditions change (most notably temperature), the symbiosis can break down (bleaching, Fig. 4), sometimes causing widespread coral mortality (reviewed in Glynn 1991; Coles and Brown 2003). Bleaching threshold and severity depends on the specific symbionts involved (Rowan et al. 1997; Glynn et al. 2001) and, after bleaching has occurred, different taxa of zooxanthellae might dominate the intracolonial symbiont community than before the disturbance (Baker 2001; Glynn et al. 2001).

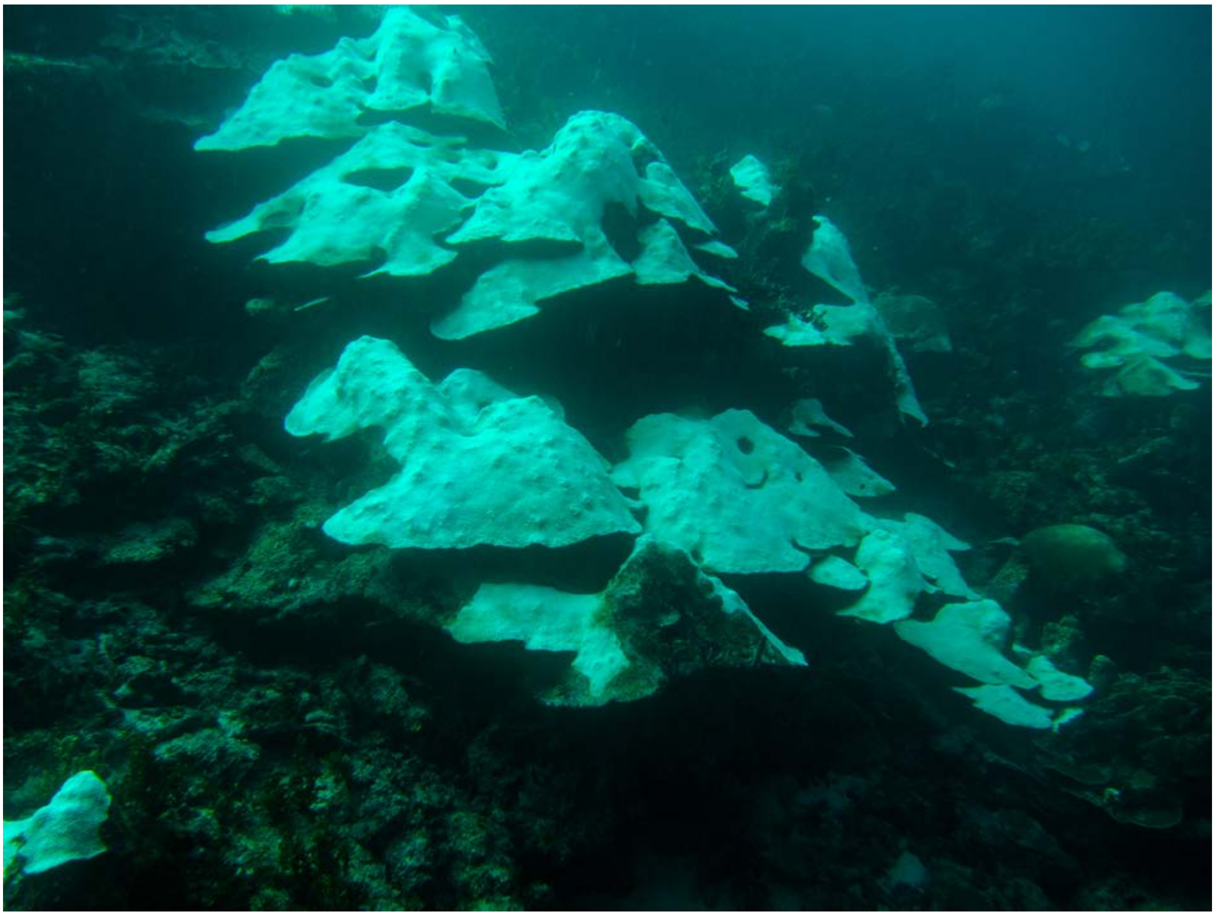

Figure 4. Bleached colony of Montastra faveolata. Because the zooxanthellae have disappeared from the colony the underlying white skeleton is visible through the living tissue. In the lower left part of the colony a part of the tissue has died.

Differential performance of coral colonies in varied environments implies that there may be sufficient inherited variability in advantageous traits (either of the host or symbionts) for selective breeding programs to improve the performance of the species in changing environmental conditions (Baums 2008). Conversely, this indicates that some genets may be maladapted for environments they may be transplanted to during restoration efforts (Baums 2008). Consequently, careful site selection and matching are needed to optimize transplantation success.

Results from a study in Bonaire with transplanted staghorn corals (*Acropora cervicornis*) indicate clear genetic differences (either symbiont or host related) in performance of the different genotypes used in restoration (Boomstra 2014). Selecting for certain genotypes will have strong effects on restoration success.

4 General description of three main restoration methods

4.1 Coral gardening (asexual restoration method)

At present, one of the most commonly used coral propagation and restoration methods is "coral gardening" (Rinkevich 1995, Bowden-Kerby 2001, Epstein et al. 2003, Shafir et al. 2006, Shafir and Rinkevich 2008, Shaish et al. 2008, Young et al. 2012). This method, adapted from terrestrial silviculture, consists of removing a limited amount of tissue and skeleton (from a few polyps to small branches) from healthy wild coral populations and propagating an initial stock within *in situ* or *ex situ* coral nurseries. In the nurseries the small coral fragments are protected from overgrowth by algae and predation. By utilizing the natural process of asexual reproduction through fragmentation, nurseries may provide a source of wild corals to be used for population enhancement. Once the corals grow large enough, they are clipped again to produce more corals or transplanted onto the reef. Nursery-grown colonies produce a stock of corals which can then be transplanted to degraded reefs (Rinkevich 1995 and 2005, Epstein et al. 2003, Soong and Chen 2003). This coral gardening methodology, in which coral colonies or fragments are grown in underwater nurseries and then transplanted back onto degraded reefs, was initially developed in the Indo-Pacific and Red Sea regions, and in recent years has been increasingly implemented throughout the Caribbean (Young et al. 2012).

In the Caribbean Netherlands Buddy Dive resort on Bonaire is now supporting this form of coral restoration following an adapted protocol of the Coral Restoration Foundation (Nedimyer et al. 2011).

Figure 5. Coral fragments growing on a tree-like structure in front of buddy dive resort on Bonaire.(Photo L.Becking)

4.2 Larval seeding (sexual restoration method)

Recent advances in coral reef science have made it possible to collect large amounts of coral eggs and sperm in the field with subsequent fertilization in the lab where coral larvae are then reared till they are fit to settle on natural or artificial settlement plates (Rinkevich 1995). The coral recruits are grown in aquaria until a certain size after which they are transplanted to the reef. This restoration technique received much attention when public aquariums and research institutions came together in two European projects SECORE and CORALZOO (Petersen et al. 2006, Osinga et al. 2012). The SECORE and CORALZOO projects matched scientific knowledge with the knowledge present in large aquariums in order to stimulate the raising of corals in aquaria and potentially use the raised corals in restoration projects. Since the discovery of coral spawning (Harrison et al. 1984), research has gradually expanded its knowledge on the timing of coral spawning and the rearing of larvae in aquaria. Keeping corals alive in aquaria however is still a difficult task. In a review of coral restoration projects in the Caribbean and Western Atlantic, Young et al. (2012) list 41 recent restoration projects of which only two projects use sexual recruits.

At present there is research on larval seeding at CARMABI in collaboration with SECORE and the University of Amsterdam (Chamberland et al. 2013).

Figure 6. Photo of juvenile Acropora coral (A. tenuis, pacific) from www.secore.org (Photo D. Petersen).

Presently, most emphasis has been on collecting gametes from *Acropora palmata* and to a much lesser degree from *A. cervicornis*. A. palmata is still relatively easy to find in many places in the Caribbean while *A. cervicornis* was almost extinct after the white band disease had passed through the Caribbean. The collected gametes are fertilized in the lab and after some time the larvae that develop will settle on provided settlement structures. Settlement and survival of larvae in aquaria depend on many variables (e.g. light, water flow, settlement plate material, nutrient concentration) and this may be one of the reasons why this method has not yet gained a large amount of followers. Furthermore, mortality is high in the initial phases of coral recruitment leading to high losses at the start of the experiment. This method has much potential for rearing other species than the two *Acropora*.

4.3 Reef Balls

Reef balls are an example of artificial structures, also called artificial reefs, designed to provide shoreline protection and prevent beach erosion while also forming substrate for natural recruitment and attachment of benthic organisms such as corals (Young et al. 2012). As such, reef balls do not restore a coral reef *per se*, but they provide a reef-like structure. The rationale behind Reef Balls is that it may form the basis for a natural coral reef community to develop if given enough time, or on which to plant out nursery reared colonies. However, this is still a topic of debate and this will be discussed in the following sections of Chapter 6. The number of participants of the Young et al. (2012) questionnaire using artificial structures was less than those using coral gardening, though more than larval seeding and electrolysis. A few examples of other artificial structures are; tyres, shipwrecks, cars, airplanes, PVC, concrete blocks or sculptures, rubble, boulders, steel, metal etc. Baine, 2001)

The Reef ball foundation distinguishes two basic restoration types: re-stabilization and propagation. Re-stabilization concerns the stabilization and reattachment of adult coral colonies that are broken off or are damaged to an alternative substrate, such as an artificial reef module. Propagation is the removal and replanting of small coral fragments (Reef Ball Foundation, 2008). The step-by-step Reef rehabilitation protocol of the Reef Ball Foundation mentions fragmentation methods per coral species.

Figure 7. Picture of reef ball with soft and hard corals artificially attached to it (from www. Reefball.org).

Reef balls are often used in shallow water where corals do not easily settle because there is scouring of sand and where reef balls can provide substrate for coral recruits to settle on. However, depth, temperature, and water movement are all strong selective forces for coral settlement and survival and these are often far from optimal. Bachtiar and Prayogo (2010) expected that the roughened surface of the reef balls should provide more stability for coral recruits compared to ordinary concrete for instance. However, the effectiveness of reef balls compared to other artificial substrates is not yet clearly defined. It is expected that reef balls face the same challenges that other artificial reef structures such as; slow development, poor control of the community development, limited knowledge and prediction ability, reduction of larval supply from natural reefs, attraction of organisms from natural reefs rather than production, possible adverse effects on neighbouring natural reefs, promotion of common/dominant species (Abelson, 2006).

Numerous Reef Ball projects have been carried out on the islands of the Bahamas, Barbados, Bonaire, Cayman islands, Curacao, Dominica, Dominican Republic, Jamaica, Saint Vincent and the Grenadines, St. Maarten, Puerto Rico and Turks and Caicos Islands (www.reefball.org). The number of scientific publications on the efficacy of the reef ball structure however is limited or monitoring reports are not readily available, making it difficult to determine the suitability of the reef ball method for coral reef restoration. Available scientific papers on reef ball efficacy are mostly studies on fish populations (e.g. Osenberg et al. 2002, Sherman et al. 2002, Neves dos Santos 2010, Folpp et al. 2013) and one on coral recruitment (Bachtiar and Prayogo 2010). However, these publications focus predominantly on areas outside of the Caribbean, such as Australia (Folpp et al. 2013), Brazil (Neves dos Santos 2010), and Indonesia (Bachtiar and Prayogo, 2010). Focus of reef balls research is often more on an increase of fish biomass than on the restoration of a coral reef. Research on reef ball efficacy for coral re-establishment should be considered a knowledge gap.

Reef balls in themselves are not enough for coral restoration. In the case of coral recruitment, reef balls are a means to provide stable substrate for coral recruits to settle on. The presence of coral recruits and good environmental conditions are still essential. Current reef ball practices may use a combination of reef balls and asexual restoration by attaching coral fragments to the reef ball in order to speed up the process (e.g. Fig. 7).

5 Comparison of restoration methods

A successful coral restoration project is determined by:

- the increase in biomass of corals;
- their reproductive rates (sexual and asexual);
- their associated fauna (e.g. fish).

There are no studies that compare the coral restoration techniques in a consistent manner, making a true comparison challenging.

The propagation of *Acropora* fragments generally has a high rate of success, 63%-95% survival documented (Young et al. 2012). Additionally, increased survival (86%-97.5%) and coral growth (up to 21.0 cm^{-1}), as well as reduced predation have been documented when propagating fragments on suspended mid-water line nurseries, which were used in 42% of projects reviewed by Young et al. (2012). High survivorship (>70%) of coral fragments was found within coral nurseries during the first year of propagation (Young et al. 2012). Mortality was often due to localized mostly natural impacts such as storm damage or other disturbances such as temperature anomalies, predation or poor water quality (Quinn and Kojis 2006). Densely clustered corals raised in a nursery may be more vulnerable to disease outbreaks, and preventative management to reduce the risk of an outbreak may be required (Johnson et al. 2011). Various fish, snails and worms have been implicated in the transmission of disease. Removing these suspected transport vectors can reduce the potential spread of disease and increase the effectiveness of disease prevention and treatment (Johnson et al. 2011).
Boomstra (2014) finds no mortality for 60 fragments transplanted back to four sites on the reef of Bonaire and monitored during 5 weeks. During this time growth of the fragments was extremely high.

The top three concerns presented by coral restoration practitioners for the fragment stabilization or nursery phases of reef restoration activities are:
(1) the sensitivity to physical damage caused by waves and storms;
(2) vulnerability to predation, and
(3) mortality due to competition by algae and other space competitors (i.e., sponges, bryozoans, tunicates).

Survival of coral colonies is generally size-related with larger colonies having higher chances of survival (Bak and Engel 1979). Thus, it is no surprise that fragments on average have a higher survivorship than larvae. However, thousands of larvae take up very little space and they can be produced at large quantities and can have, in theory, a much higher genetic diversity than a population of fragments which is often generated from a very small number of stock colonies. Survival rates of larvae in the lab were only found in one paper (Hagedorn et al. 2009) and decrease rapidly during the first 6 days to less than 20%. When sexually derived coral recruits are kept in a midwater coral nursery, however, survival can be as high as 89% (Linden and Rinkevich 2011). More research is being conducted by CARMABI on Curaçao (http://www.researchstationcarmabi.org) and SECORE (www.secore.org).

5.1 Survival of transplants on the restoration sites

The art of restoration is still under development and Young et al. (2012) report high variability in the level of success of restoration activities throughout the Caribbean. Excessive mortality was often induced by natural causes (storms, bleaching, predation) indicating that carefully choosing restoration sites is crucial to minimize the chances of extensive mortality to the new recruits.

We consider as transplants, the coral colonies that have been placed on the restoration site. There is little information on the long-term survival of fragments which would enable the comparison of not only the success of the transplantation, but also the suitability of the environment into which the fragments were restored. Coastal development and population increase has changed water quality throughout the Caribbean. Thus, survival of colonies on the long run may nowadays be very different from what it once was. Success of the transplants will be location specific. Boomstra (2014) studying growth and survival of fragments in nurseries and on transplant sites found no difference in growth rates between four different sites on Bonaire. Survival during the first 5 weeks after transplantation was 100% and growth rates were with an average of 13.9cm per year among the highest ever recorded for *Acropora cervicornis*.

While some projects (12.5%) focus on simple fragment stabilization or transplantation of corals onto natural reefs after physical disturbances such as ship or storms (Garrison and Ward 2008, Bruckner et al. 2009), almost 60% of projects outplant nursery-grown corals onto degraded reefs or artificial structures as a final restoration step (Johnson et al. 2011).

Data on the survival of transplants originating from sexual reproduction are sparse. In Curaçao survival appears to be around 11% after one year but it may be influenced strongly by recruit size (Chamberland 2013). Most sexually reared transplants have been used in coral aquaria and only very limited data were found on transplant survival in the field. Aquaria are generally positive about survival of larvae but corals survive only for short periods life in aquaria (personal observation E.M.).

Due to the limited amount of monitoring data and information on the progress of the reef ball projects concerning coral restoration, it is difficult to determine the survival after transplantation and growth rate. Only few monitoring reports concerning a coral rehabilitation project on Curacao (PortoMarie) are available (Van den Bulck 2002, 2004). After 33 months, 28% of the original coral plugs (*Faviidae sp., Acroporidae sp., Poritidae sp. , Meandrinidae sp. , Pocilloporidae sp. , Astrocoeniidae sp., Siderastrea sp., Gorgonacea sp.*) still survived. Staghorn coral (*Acropora cervicornis*) had a survival rate of 36% and 50% after respectively 33 and 21.5 months. This coral species also showed the greatest growth compared to other corals after approximately 2 years (2002 – 2004) (Van den Bulck 2002, 2004). Project reports were available till 2011 and show generally downward trends for coral cover and increasing cover of algae.

Bachtiar and Prayogo (2010) examined the pattern of coral larval recruitment on reef ball structures on sandy bottom substrate, where corals did not grow naturally due to the lack of suitable substrate, at Sumbawa Island, Indonesia. After a deployment of three years, coral recruitment on thirty reef ball modules (4-12m depth) was measured. A total of 640 colonies were counted. Most coral colonies growing on the reef balls belonged to the Acroporidae (75.8% of the total recruitment). The number of coral recruits was lower at deeper waters (10 – 12m) than middle (7-9m) and shallow waters (3-6m). Whether this was the result of lower larval supply, lower larval settlement or lower post-settlement survival or a mix of these factors was not determined.

A personal observation (by EM and LB) of a reef ball project that has been running for over 10 years in Sint Maarten suggests that reef balls are often colonised by sponges and fire coral, not a hard coral, but a milleporid which prevents other hard corals from settling on the reef ball. Personal observations suggest that recruitment of corals on reef balls is almost non-existing. The reasons for this are multiple. Reef ball structures are built in waters that are often not very suitable for coral recruitment (e.g. in densely populated areas or near harbours) or in areas that did not historically have high coral cover.

5.2 Introduction of exogenous material

All current restoration techniques introduce foreign, often non-natural, material into the ecosystem. This happens mostly when fragments or sexual recruits are in some way fastened to the bottom on the restoration site or the nursery site where fragments are grown.

Within coral nurseries, *Acropora* fragments are grown on frames, ropes, cinderblock platforms, Reef Balls, floating structures, and through electrolysis processes known as the BioRock method (Young et al. 2012). In the questionnaire by Young et al. (2012) the highest-ranked outplanting (transplanting nursery-grown corals to the reef) method was securing fragments or colonies to the reef substrate using cement, epoxy, cable ties, wire, or any combination of these. This was considered more effective than wedging corals directly into holes and crevices in the reef framework, attaching corals to lines, ropes or mesh secured to the substrate, or affixing corals to nails driven into the substrate. Many studies found the use of small plastic cable ties to be a cheap, quick, and effective method for attaching corals to artificial or reef substrate (Bruckner et al. 2009, Williams and Miller 2010, Johnson et al. 2011, Garrison and Ward 2012).

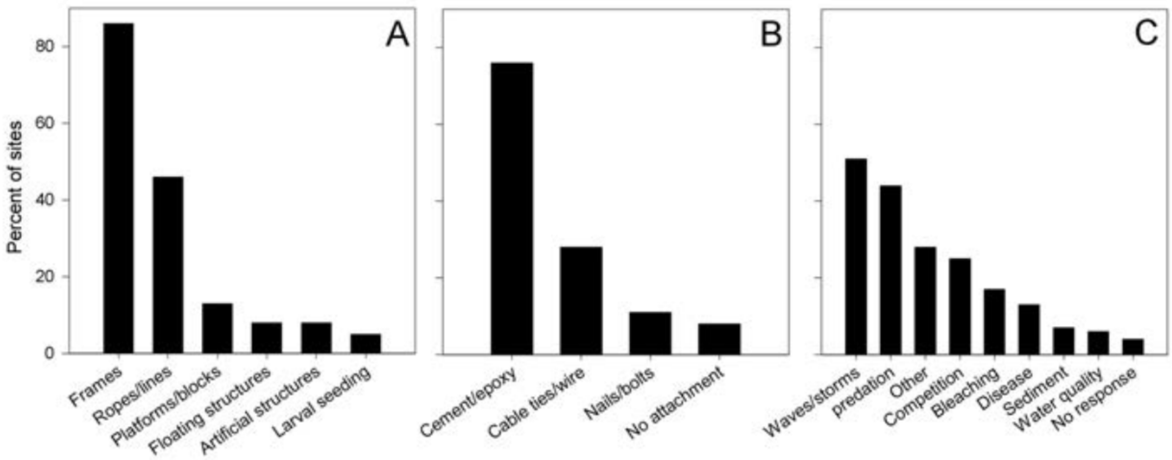

Figure 8. Percentage of Caribbean Acropora restoration sites (A) using various propagation methods,(B) utilizing various fragment attachment methods, and (C) experiencing common restoration concerns (Young et al. 2012).

With sexual restoration the aim is to generate so many settled recruits on ceramic tiles or tripods that these can then be seeded on the reef by the thousands. No further attachment of tiles/tripods is necessary as a certain amount of loss is viewed as acceptable. Generally, this means that the introduction of exogenous material is very low.

With the use of Reef balls, exogenous material is introduced into the ecosystem. Reef balls are made from mixtures of micro silica concrete. Protocols on tools and supplies, developing and deploying the reef ball modules are found at http://www.reefball.org/tm/tm/tm.htm. Studies on the effects of reef ball materials on the surrounding environment are not found. This seems to be a knowledge gap.

5.3 Indirect effects of restoration activities on the natural reef

The collection of larvae and fragments from the reef and restoration of transplants may have indirect effects apart from the the restoration goals. Often the focus is only on restoring a previous situation without giving sufficient thought to all possible consequences. There is little information available about the fate of the colonies that are used to create fragments, the so called “mother colonies”. If fragments

are harvested off larger colonies, the originating colony is damaged and this may seriously affect the survival chances of this colony. For the *Acropora* species regeneration after physical damage is high (Bak 1983) thus permanent damage to 'mother' colonies after fragmentation is likely low. For other species, however, regeneration is much slower (Meesters et al. 1992) and wounds can become infected by algae and diseases and lead to mortality. Therefore, if coral fragmentation is conducted the "mother" colony should be monitored over a long term (>12months). Many restoration projects use volunteers and sometimes part of the work is at night; this carries the risk that corals are damaged during collection and restoration activities.
The newly introduced colonies may also effect the environment into which they are introduced. They may alter local water currents and chemistry, and other organisms. *Acropora* colonies growing on the reef provide 3 dimensional structure between which fish and other animals can find shelter and protection from predators. Three D complexity has been found to increase fish diversity and biomass (Wilson et al. 2007). Because the branching Acropora species grow fast, it is likely that they will contribute relatively quickly to the 3D complexity of the reef and increase fish diversity and biomass. This is also something that has been found with reef balls and their use is often accompanied with claims stating higher fish densities. Folpp et al. (2013) found a greater fish abundance, fish species richness and diversity on artificial reefs (reef balls) than on adjacent naturally occurring reef or sand-flat in three Australian estuaries, 1,5 year after deployment. Hackradt et al. (2011) showed that variability in structural features influenced species richness and diversity, and may favour particular species.
Reef Balls may attract fish, however from a recreational divers point of view Reef balls together with rubber tyres were the least favoured artificial structures to visit during a dive, compared to for example shipwrecks, piers, cars and aeroplanes (Kirkbride-Smith et al. 2013). Aesthetics is only a concern if the area is allocated for dive tourism.

5.4 Genetic diversity

Due to the importance of preserving the genetic integrity of populations, strategies to restore damaged coral reefs should attempt to retain or increase the genetic diversity of the disturbed population (Baums 2008, Shearer et al 2009).

As far as genetic variation and coral restoration practices are concerned, three major issues need to be carefully considered:

- preventing the establishment of monocultures;
- outseeding maladapted recruits;
- introducing non-native zooxanthellae strains.

The coral gardening method of coral restoration relies upon asexual reproduction via fragmentation. Overreliance on asexual fragmentation limits the number of genetically distinct colonies. Clearly, fragments or asexual recruits from a single donor colony or even a few donor colonies are insufficient to replicate a significant proportion of the genetic diversity of any coral populations (Shearer et al. 2009). If not managed carefully there is the risk of reducing genetic variation. Symbiont diversity in *A. cervicornis* depends on the zone where the corals grow (Baums et al. 2010). There is therefore a potential of introducing non-native zooxanthellae strains (*Symbiodinium* spp) to transplantation sites with unknown ecological consequences. Coral reef restoration efforts with coral stock that has been nursed either *in situ* or *ex situ* are well underway (Shafir et al. 2006; Amar and Rinkevich 2007). The source of the transplantation stock varies. Captive environments are likely selective and this may extend to *in situ* nurseries (Shafir et al. 2006).

The larvae seeding is the most genetically diverse method and will likely represent the natural genetic diversity, but the breeding program in the lab may possibly (indirectly) be selecting for specific traits that are essential in the lab but not necessarily in the natural reef environment. This requires further research, which is part of a project by CARMABI.

In chapter 6, recommendations for genetically sound restoration projects are provided.

5.5 Feasibility and effectiveness

There is a need for low-cost and low-tech restoration methods. On a cautionary note, Ferse (2010) showed that too cheap brings no result in the long run – resulting in a net loss due to loss or resources and effort that could have been better spent elsewhere. Examples include many minimally funded transplant mitigations required during coastal construction projects but not funded, and not monitored. While policy intentions and efforts may be well intended, results of such projects are undocumented and likely contra-productive. The aim should, thus, be cost effective measures that last long-term.

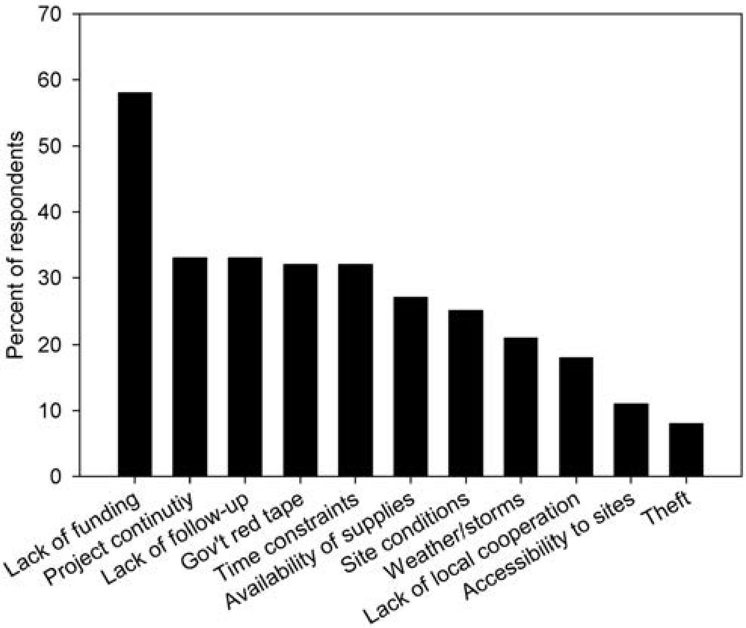

Figure 9. Results from questionnaire of Young et al. (2012): Percentage of respondents indicating common obstacles of Caribbean reef restoration projects.

Respondents of the questionnaire by Young et al. (2012) were asked to rank a variety of concerns related to reef restoration practices; their reply from high to low ranking:

(i) High financial cost;
(ii) The risk of damage to donor colonies;
(iii) manipulation of nature;
(iv) changes in genotypic diversity.

The highest ranked and most effective coral reef propagation and restoration techniques were low-tech methodologies, utilizing inexpensive and readily available materials such as wire mesh, PVC, plastic cable ties, cinder blocks, nails, fishing line, and ropes (Becker and Mueller 2001, Bowden-Kerby 2001, Herlan and Lirman 2008). This indicates that propagation and restoration activities using *Acropora* have the potential to be conducted successfully at low cost. Respondents indicated that project continuity beyond the initial funding cycle will depend on the involvement of local stakeholders outside the scientific and management community. Thus, the adoption of propagation and restoration projects by dive shop operators, resort owners, fishermen, and local communities were identified as key components to the long-term success of restoration programs.

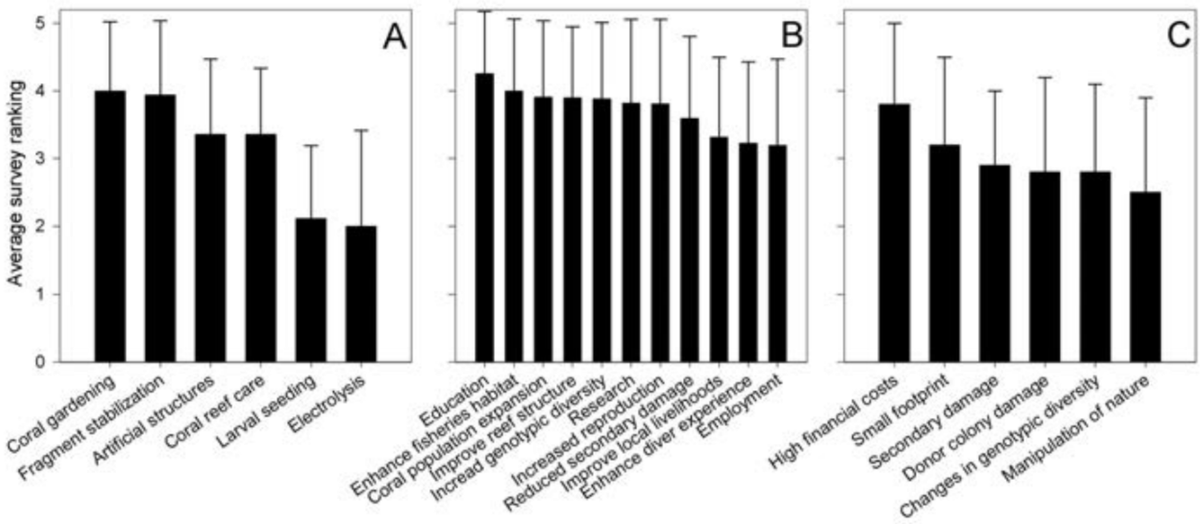

Figure 10. Mean ranking by respondents on (A) the effectiveness of various restoration methods, (B) the potential benefits of reef restoration, and (C) concerns facing coral reef restoration efforts (from Young et al. 2012).

Reef ball mold costs range from $ 719 - $ 11,448 depending on the size of the reef ball. In addition to the mold different kits and concrete additives need to be purchased (Reef ball Foundation, 2007). If the aim is to restore coral reefs, then it is not cost-effective to use Reef Balls, considering that Reef Balls have not shown to increase coral cover. If the aim is to increase fish abundance, then reef balls or other artificial reef structures (e.g. shipwrecks, jetties, rock mounds) could possibly be considered.

The larval seeding method may have a high initial cost and require more time before it’s effect can be measured (before adult corals are added to the reef), yet this method may be the most sustainable with the most resilient corals. The actual cost-effectiveness and success rate of the larval seeding methods requires further study.

5.6 Summary overview of restoration techniques

Characteristics	**Larvae**	**Gardening**	**Reefballs**
Foreign material	Low	Moderate	High
Survival in nursery/lab	Still being studied	High	*Not applicable*
Survival in reef	Still being studied	High	Low
Effects on natural reefs	Needs study	Needs study	Positive for fish abundance. Little to no effect for corals
Genetic diversity	Good	Potentially low	Potentially low
Costs and feasibility	High	Moderate	Low effectiveness for corals

6 Setting up a genetically sound restoration project

Reef restoration goals are mostly directed toward re-establishing communities and preserving biodiversity. Baums (2008) has provided some guidelines on how to create self-sustaining populations that resemble native reefs in their genetic composition and complexity. During the design stages of (sexual and asexual) restoration projects, the most immediate question is the source of the coral propagules. Because of the prevalence of population subdivision and potentially ecotypes in corals, source and recipient sites should be physically close and similar in environmental conditions (i.e. depth, sediment load).

In general, scleractinian coral populations are genetically diverse and restoration methods utilizing too few clonal genotypes to re-populate a reef will diminish the genetic integrity of the populations (Shearer et al. 2009). Most natural coral populations are not monoclonal and restoration efforts should avoid producing monoclonal populations. By increasing the number of distinct parent genotypes at local scales through propagation and transplantation of nursery grown fragments, sexual reproduction and recruitment are expected to have higher success rates ultimately aiding in the natural recovery of the species (Johnson et al. 2011). A study by Shearer et al. (2009), which aimed to provide a generalized estimate of genetic variation (in terms of allelic richness) of natural scleractinian coral populations, indicated that coral restoration strategies using 10–35 randomly selected local donor colonies will retain at least 50–90% of the genetic diversity of the original population (Shearer et al. 2009). We do, however, strongly recommend to make a site-specific assessment of genetic diversity for localities in the Dutch Caribbean. The source colonies that are now being used by the coral restoration foundation Bonaire are collected from 10 colonies in the field that were spread all over the island (including the windward side). Thus their genetic diversity may well be large, but at present we do not know for sure. Boomstra (2014) indicates that growth differences between the fragments indicate a genetic component. The natural genetic diversity of the coral populations surrounding the islands , i.e. the natural number of genotypes (of coral and symbionts), should be compared to the number of genotypes in the nurseries.

Different populations can differ fundamentally in reproductive character and may respond differently to environmental change (Baums et al. 2006). Their difference in strategy may also be a result of adaptation to local environmental factors. Thus, environmental and geographic diversity should be considered in coral restoration projects. Lundgen et al. (2013) presents two novel molecular strategies to identify genetic markers in the genera *Acropora* and *Pocillopora* that are correlated to environmental gradients and environmental stress. Differential performance of coral colonies in varied environments implies that there may be sufficient inherited variability in advantageous traits (either of the host or symbionts) for selective breeding programs to improve the performance of the species in changing environmental conditions (Baums 2008). Currently, *Acropora* fragments of different origin are mixed when they are transplanted back to the reef with the intention to maximize genetic diversity, however, since no assessment has been made, the efficiency of this strategy is unclear.

While even the largest reef restoration projects are minimal in comparison to the scale of natural processes during a successful sexual recruitment event, establishing multiple small, genetically diverse populations that will, in time, become sexually reproductive can contribute to species recovery, especially in areas of significant parent population declines (Baums et al. 2005, Vollmer and Palumbi 2007). Therefore, by strategically restoring populations to fill spatial gaps in species distribution, small reproductive populations may have the potential to significantly contribute to the overall success of gamete fertilization and sexual recruitment of *Acropora* populations.

6.1 Recommendations for restoration techniques to retain genetic variation

- What is the source of the coral propagules? Source and recipient sites should be physically close and similar in environmental conditions (i.e. depth, sediment load).
- Genotypes for every coral brought into the nursery should be determined and accurate records should be kept during fragmentation so that the genotype of every coral is known.
- Local genetic variation of the natural populations in the reefs surrounding the islands of the Dutch Caribbean should be assessed to determine source populations and numbers of genets required in the nurseries.
- In the absence of knowledge of the local genetic variation, coral restoration strategies should use at least 35 randomly selected local donor colonies to retain at least 90% of the genetic diversity of the original population (Shearer et al. 2009).
- Symbiont diversity in *Acropora* depends on the zone where the corals grow (Baums et al. 2010). Nurseries should thus be established in multiple habitat types to maintain a range of holobiont types.
- Differential performance of genotypes in captive environments can be assessed using molecular tools to differentiate among genotypes and track them and their offspring over time (Baums 2008).
- Genetic variation should be maximized within an outplant/restoration site to increase chances of successful cross-fertilization and decrease chances of inbreeding. When spacing sexual and asexual propagules, the mating system of the coral species should be taken into account so that stands of only one sex in gonochoric species or stands of only one genet in self-incompatible hermaphrodites are avoided.
- Following general IUCN guidelines (IUCN 2002), restoration efforts should, at the very least, carefully track ramets and genets used in transplantation efforts to further assess performance of genets and the consequences of transplantation on survival of coral communities.

7 General recommendations

Conservation of corals through nurseries and larval seeding is still impaired by knowledge gaps. A number of studies are suggested:

- Identification of genotypes/ecotypes in Bonaire (and Dutch Caribbean). To provide a general estimate of genetic variation of scleractinian coral populations.
- Determine optimal spatial layout of the transplants to optimise genetic diversity.
- Monitor long term effect of restoration activities on source corals.
- Monitor survival, growth and indirect effects of transplants.
- Limit the introduction of exogenous material on the reefs as much as possible.
- Assess effect of nurseries on fish abundance.
- Determine optimal manner to attach transplants to reef with minimal damage/disturbance to the reef.
- Assess success rate and applicability of larval seeding methods.
- Compare cost-effectiveness and success rate of the different types of reef restoration techniques.
- Explore the use of other species beside staghorn and elkhorn coral.
- Develop restoration techniques to mitigate the effects of diseases.
- Ensure restoration project continuity beyond the initial funding cycle through involvement of local stakeholders outside the scientific and management community (e.g. dive shop operators, resort owners, fishermen).

8 Quality Assurance

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 124296-2012-AQ-NLD-RvA). This certificate is valid until 15 December 2015. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Fish Division has NEN-EN-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 1th of April 2017 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

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10 Justification

Rapport ~number~
Project Number: ~number~

The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of IMARES.

Approved: A.O. Debrot
Researcher

Signature:

Date: 1 Sept 2014

Approved: F. Groenendijk
Head maritime Department

Signature:

Date:

11 Appendices

Appendix 1. Glossary genetic terms

(from http://evolution.berkeley.edu/evolibrary/glossary/glossary.php)

Allele: One of the versions of a gene that may exist at a locus. For example, the pea color locus may have either the yellow allele or the green allele.

Ecotype: A subdivision of an ecospecies consisting of a population that is adapted to a particular set of environmental conditions.

Gene: The unit of heredity. Generally, it means a region of DNA with a particular phenotypic effect. Technically, it may mean a stretch of DNA that includes a transcribed and regulatory region.

Genotype: The set of genes an organism has. Sometimes, genotype refers to the entire genome of an organism and sometimes it refers to the alleles carried at a particular locus.

Genetic variation Loosely, a measure of the genetic differences there are within populations or species. For example, a population with many different alleles at a locus may be said to have a lot of genetic variation at that locus. Genetic variation is essential for natural selection to operate since natural selection can only increase or decrease frequency of alleles already in the population.

Genetic diversity: variation in genetic material from an individual, population, or species. This can be characterised by multiple statistics, typically calculated at the locus level.

Genet: an individual that develops from a zygote and is, therefore, a product of sexual reproduction

Genome: All the genetic information an organism carries

Holobiont: the host plus all of its symbiotic microorganisms

Locus: The place in the DNA where a gene is located. For example, the pea color locus is the place in a pea plant's DNA that determines what the color of the peas will be. The pea color locus may contain DNA that makes the peas yellow or DNA that makes the peas green — these are called the yellow and green alleles.

Ramet: an individual that develops from a genet or another ramet and is a product of asexual reproduction. A Ramet is an exact genetic copy of its parent colony, and is thus considered a clone. Clones behave exactly the same under equal circumstances, which means they are equally strong, but also equally weak as their parent colonies.

Population genetics: The study of how allele and genotype frequencies in a population change over time and across space.

Population: a group of individuals of the same species, usually living close to one another and that interbreed with one another and do not breed with other similar groups. Depending on the organism, populations may occupy greater or smaller geographic regions.

Gene flow: The movement of genes between populations. This may happen through the migration of organisms or the movement of gametes

Gene pool: All of the genes in a population. Any genes that could wind up in the same individual through sexual reproduction are in the same gene pool.

Zooxanthellae: Most reef-building corals contain photosynthetic algae, called zooxanthellae, that live in their tissues. The corals and algae have a mutualistic relationship. The coral provides the algae with a protected environment and compounds they need for photosynthesis. In return, the algae produce oxygen and help the coral to remove wastes. Most importantly, zooxanthellae supply the coral with glucose, glycerol, and amino acids, which are the products of photosynthesis. (http://oceanservice.noaa.gov/education/kits/corals/coral02_zooxanthellae.html)

Appendix 2. Assignment

In view of reef restoration efforts on Bonaire (http://www.crfbonaire.org/), the Dutch Ministry of Economic Affairs (EZ) has requested IMARES to review currently used coral restoration methods. The methods concerned are:

a) coral gardening (an asexual method using coral fragments)
b) the use of settled larvae (sexual restoration), and
c) the use of reef balls (artificial substrate).

The assignment had the following main objectives:

- To determine the possible risks
- To assess the cost-effectiveness of the restoration methods involved.
- To determine the necessary conditions and monitoring for an effective and sustainable application of a restoration method.